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the improvement wherein the affinity particles suitable for binding said molecule of interest in step (a) are incubated in the presence of a detergent in an amount sufficient to reduce loss of affinity particles during any subsequent separation step, in comparison to the same method performed in the absence of detergent; and

wherein any of the steps (b), (c), (d), (e) if present, and (f) if present may optionally be also performed in the presence of detergent[, wherein the use of detergent is sufficient to reduce loss of particles during any separation step, in comparison to the same method performed in the absence of detergent].

REMARKS

Applicants have amended the claims to clearly cover separation and isolation methods using affinity particles in which a detergent is specifically employed to reduce loss of the affinity particles and, thereby, improve particle handling and yields of molecules isolated by such methods (see, e.g., Table 1, p. 21; Table 2, p. 22; Table 3, p. 23; Table 4, p. 25; and Table 6, p. 29 in the specification). In particular, Applicants have amended independent Claims 1, 2, 33, and 34 (and thereby claims depending therefrom) to cover separation and isolation methods of the invention using affinity particles and a detergent in at least one step of the procedure to reduce particle loss. Support for these amendments is found in the specification (see, e.g., p. 7, lines 14-25; p. 10, lines 22-24; and p. 16, lines 26-30). Applicants have also similarly amended independent Claims 64 and 66 (and, thereby, claims depending therefrom) which specifically use magnetic affinity particles. Support for the amendments to Claims 64 and 66 are found in the specification (see, e.g., p. 19, lines 1-6; p. 27, lines 10-17). Accordingly, the amendments add no new matter.

Applicants have also amended Claims 18, 31, 32, 49, 50, 62, and 63 to correct an inadvertent typographical error. Specifically, in the description of various nonionic detergents useful in the methods of the invention to reduce particle loss, the incorrectly spelled term "polyoxyehtylene" is corrected to "polyoxyethylene". Support for these amendments is found in the specification (see, e.g., p. 13, lines 15-17). Accordingly, the amendments add no new matter.

Entry of the above amendments is respectfully requested.

The Invention

The invention is based on Applicants' discovery of how to reduce particle loss in affinity procedures in which affinity particles are manipulated and subject to loss during separation steps.

According to the invention, adding detergent to one or more steps in an affinity procedure prior to and/or contemporaneously with a step in which the affinity particles are manipulated (e.g., collected, separated, or washed) will reduce loss of the affinity particles compared to the same procedure carried out without the benefit of detergent treatment (see, e.g., Examples section, pp. 20-29 of the specification). Hence, Applicants' claimed invention improves all prior art affinity procedures in which affinity particles are manipulated in the absence of a detergent, which as Applicants have demonstrated invariably results in particle loss and, thereby, reduced and inconsistent yields.

Rejections Under 35 U.S.C. § 102(e)

In Paper No. 6, the Examiner rejected Claims 1-4, 9, 13-18, 19, 23, 24, 31-35, 44-50, 54, 55, and 62-66 under 35 U.S.C. § 102(e) as anticipated by U.S. Pat. No. 5,942,391 (issued August 24, 1999, "Zhang"). The Examiner also rejected Claims 1-4, 9, 13-17, 20, 33-35, 44-46, 48, 49, and 64-66 under 35 U.S.C. § 102(e) as anticipated by U.S. Pat. No. 5,466,577 (issued November 14, 1995, "Weisburg"). In particular, the Examiner was of the view that each of Zhang and Weisburg demonstrated each step in the claimed methods for isolating or separating a molecule and for specific embodiments wherein the molecule to be isolated or separated may be a nucleic acid. Applicants respectfully traverse the rejection for the reasons explained below.

Zhang

The Zhang patent describes methods for detecting a target nucleic acid from a pathogenic microorganism or from patients with genetic diseases or cancer (see, e.g., col. 3, lines 9-16; col. 5, line 61-col. 6, line 20 of Zhang). The methods of Zhang use multiple nucleic acid probes, including a "capture probe", which is attached to the surface of paramagnetic particles and which binds to a target nucleic acid molecule (see, e.g., col. 3, lines 52-59; Figure 1 of Zhang).

Nowhere does Zhang teach or suggest how to reduce loss of affinity particles to improve an affinity separation or isolation procedure for a molecule of interest. A person of ordinary skill in the art who reads Zhang is never informed of how to reduce loss of affinity particles when used in a separation or isolation procedure. Zhang does not provide any example that shows the benefit of using detergent in affinity methods as taught and demonstrated in Applicants' specification (see, e.g., Examples section, pp. 20-29 of the specification) or as recited more clearly in the amended claims.

Weisburg

The Weisburg patent describes nucleic acid probes that hybridize to specific target sequences in the 16S ribosomal RNA of *Borrelia* bacterial species, such as *B. bugdoferi*, the

etiological agent of Lyme's Disease. Weisburg also describes the use of such probes to detect *Borrelia* target nucleic acid in dot blots (see, e.g., Example 1, col. 6, line 66-col. 7, line 31 of Weisburg) and sandwich hybridization schemes where a "capture" probe binds a target sequence and a "detector" probe signals the binding of the target sequence (see, e.g., Example 2, col. 7, lines 35-62). Example 3 of Weisburg describes a sandwich hybridization protocol to diagnose Lyme's disease from blood in which the capture probe is linked to a magnetic particle (see, col. 8, lines 6-12). However, nowhere does Weisburg teach or suggest use of a detergent in a separation or isolation method that uses affinity particles in order to reduce loss of affinity particles in subsequent steps of the procedure.

The Examiner argues anticipation of Applicants' claimed invention by finding specific steps in the procedure of Zhang or of Weisburg that include a detergent. For example, the Examiner stated that Zhang describes the use of a buffer containing a detergent to wash paramagnetic beads containing nucleic acid hybrid molecules on their surface (col. 40, line 6 of Zhang). In Weisburg, the Examiner relied on a description that SDS could be used in a buffer to lyse cells in a procedure to isolate RNA using magnetic beads derivatized with oligo-thymidine residues (col. 8, lines 3-11 of Weisburg). Neither of the examples in Zhang or Weisburg recognize the problem of, or provide a solution for, reducing particle loss in affinity procedures in which the particles are manipulated in the absence of a detergent. Accordingly, neither Zhang nor Weisburg anticipate the claimed improved methods of Applicants' invention.

As noted above, Applicants have amended the claims to more clearly cover the improved methods of the invention for reducing particle loss in affinity procedures that involve manipulation of affinity particles. Neither Zhang nor Weisburg describes such an improvement in prior art affinity procedures. Accordingly, Zhang and Weisburg are not effective references for anticipating Applicants' improved methods as presently claimed.

In view of the above comments and the amendments to the claims, Applicants respectfully request that the Examiner reconsider and withdraw the rejections under 35 U.S.C. § 102(e).

Rejections Under 35 U.S.C. § 103(a)

In Paper No. 6, the Examiner rejected Claims 1-4, 9, 13-17, 20, 25, 26, 33-35, 44-49, 56, and 64-66 under 35 U.S.C. § 103(a) as obvious over Weisburg. The Examiner also rejected Claims 1-19, 23, 24, 31-50, 54, 55, and 62-66 as obvious over Zhang in view of U.S. Pat. No. 5,646,016 (issued July 8, 1997, "McCoy"). Claims 1-4, 9, 13-19, 21, 23, 29-35, 44-50, 52, 54, 55, and 60-66 were rejected by the Examiner as obvious over Zhang in view of U.S. Pat. No.

5,798,442 (issued August 25, 1998, "Gallant"). Finally, Claims 1-4, 9, 13-19, 22, 27-29, 31-35, 44-50, 52-55, and 58-66 were rejected by the Examiner as obvious over Zhang in view of U.S. Pat. No. 4,009,213 (issued February 22, 1977, "Stein"). For the reasons discussed below, Applicants respectfully traverse these rejections.

As noted above, Applicants have amended the claims in the application to more clearly claim Applicants' improvement over prior art methods of using affinity particles, which improvement employs detergent to reduce particle loss that otherwise can occur during manipulation of the affinity particles.

As noted above, the primary references Zhang and Weisburg describe nucleic acid probes and their use to detect target nucleic acid molecules related to various diseases.

Weisburg describes nucleic acid probes that hybridize to specific sequences found in the 16S ribosomal RNA of *Borrelia* bacterial species, especially *B. burgdorferi*, which causes Lyme's Disease. Weisburg describes the use of such probes for the clinical diagnosis of Lyme's disease in humans and other animals (see, e.g., col. 3, lines 51-67). Such probes may be linked to a magnetic particle as described in Example 2 of Weisburg. However, nowhere does Weisburg contemplate, teach, or suggest Applicants' claimed methods for improving affinity methods by using detergent to reduce particle loss that otherwise occurs during manipulation of the affinity particles. Weisburg does not even recognize the problem of particle loss in various prior art affinity procedures or provide any suggestion or motivation to be combined with any of the other references cited by the Examiner to solve the problem of particle loss.

Based on the above comments and the amendments to the claims, Applicants respectfully submit that it is clear that Weisburg, which is directed to methods of detecting specific nucleic acid molecules, does not make Applicants' claimed improvements over prior art procedures *prima facie* obvious. Accordingly, Applicants respectfully request that the Examiner reconsider and withdraw the rejections based on Weisburg under 35 U.S.C. § 103(a).

The Basis for Combining Zhang with Secondary References

The Examiner relied on Zhang as a primary reference in combination with each of McCoy, Gallant, and Stein to reject claims as *prima facie* obvious under 35 U.S.C. § 103(a). At the outset, Applicants note the well-established standard regarding combining references as a basis for rejecting claims as *prima facie* obvious. Obviousness cannot be established using Applicants' own disclosure as a guide to merely selecting and reconstructing the claimed invention from elements in the prior art. The patent law is clear:

> "Obviousness cannot be established by combining the teachings of the prior art to produce the claimed invention, absent some teaching or suggestion supporting the combination. Under section 103, teachings

> of references can be combined *only* if there is some suggestion or incentive to do so." (*ACS Hospital Systems, Inc. v. Montefiore Hospital*, 732 F.2d 1572, 1577; 221 USPQ 929, 933 (Fed.Cir. 1984), citations omitted, emphasis in original).

Evidence of a suggestion or motivation to combine references may be found in the references themselves or in the knowledge of one of ordinary skill in the art. *In re Fine*, 837 F.2d 1071, 1074, 5 USPQ2d 1596, 1598 (Fed.Cir. 1988); *In re Jones*, 958 F.2d 347, 351, 21 USPQ2d 1941, 1943 (Fed.Cir. 1992). The motivation to combine may derive from many sources, however, the range of possible sources that may serve as evidence for a motivation to combine references "does not diminish the requirement for actual evidence. That is, the showing [of a motivation to combine] must be clear and particular." *In re Dembiczak*, 50 USPQ2d 1614, 1617, 1999 WL 246572 (Fed.Cir. 1999).

Omitting a particular statement of the suggestion or motivation to combine prior art references to make a claimed invention simply amounts to hindsight reconstruction based on an inventor's own teachings. As the Court of Appeals for the Federal Circuit noted in *In re Dembiczak*:

> "Combining prior art references without evidence of such a suggestion, teaching, or motivation simply takes the inventor's disclosure as a blueprint for piecing together the prior art to defeat patentability -- the essence of hindsight." *In re Dembiczak*, 175 F.3d 994, 999; 50 USPQ2d 1614, 1617; 1999 WL 246572 (Fed.Cir. 1999).

It is contrary to the law to reject claims as obvious by using hindsight reconstruction, in which elements are merely gathered from the references using the framework provided by an inventor's disclosure:

> "One cannot use hindsight reconstruction to pick and choose among isolated disclosures in the prior art to deprecate the claimed invention." *In re Fine*, 837 F.2d 1071, 1075; 5 USPQ2d 1596, 1600 (Fed.Cir. 1988).

Zhang describes methods employing multiple probes to detect a target nucleic acid molecule, either by amplifying copies of selected sequences in the target molecule ("RAM") or by using amplified numbers of signal probes to emit a detectable signal ("HSAM") to indicate that a target nucleic acid molecule has been bound by a probe for the target (see, e.g., col. 6, lines 4-8, Figs. 1 and 10 of Zhang). One type of probe is a "capture-amplification" probe described by Zhang which may be linked to a paramagnetic particle (see, e.g., col. 7, lines 25-30; Figs. 1 and 10 of Zhang). However, Zhang does not recognize in any way the problem affinity particle loss

as addressed and solved by Applicants' claimed improved methods. Furthermore, Zhang does not provide a suggestion or motivation to be combined with any other reference cited by the Examiner to make Applicants' claimed improved methods. Accordingly, Zhang alone cannot render Applicants' claimed methods obvious under 35 U.S.C. § 103(a).

The Examiner sought to make Applicants' claims *prima facie* obvious based on the combination of Zhang with any of several other references. However, as shown in detail below, not only do the other references relied on by the Examiner fail to teach or suggest Applicants' claimed improvement in affinity procedures, but they also fail to provide the required evidence of a motivation to be combined with Zhang to suggest Applicants' claimed invention. Moreover, as is made clear below, even if the references are considered together for the sake of argument, the result is an odd combination of disparate methods, not a disclosure of Applicants' claimed invention that advances the art of affinity procedures by reducing particle loss.

Zhang and McCoy

The secondary reference, McCoy, describes compositions and methods for enhancing expression of a protein of interest by constructing recombinant DNA molecules encoding a fusion protein comprising the protein of interest fused to thioredoxin (see, e.g., col. 3, lines 34-43 of McCoy). The fusion proteins of McCoy are also mutated to contain at least one "patch" of two or more metal chelating histidine residues, preferably in the thioredoxin portion, that enhances the metal binding ability of the fusion protein (see, e.g., col. 11, lines 9-58 of McCoy). The enhanced metal binding ability of the fusion protein is exploited during purification of the fusion protein by using a standard metal ion affinity matrix or resin (see, e.g., col. 16, lines 32-51). However, nowhere does McCoy teach or suggest Applicants' methods of reducing particle loss in affinity procedures that involve manipulating affinity particles. Applicants' claimed methods are improved methods over the standard prior art methods that do not use detergent, e.g., as practiced in McCoy.

The Examiner appears to be of the view that McCoy provides motivation to be combined with Zhang:

> "McCoy further provides motivation as he states, 'There is provided another novel method for increasing the production of soluble recombinant proteins (column 4, lines 22-24)'. An ordinary artisan would have been motivated by the express statement of McCoy to utilize the histidine patch containing fusion proteins of McCoy et al in the method of Zhang et al in order to achieve the express advantage of an improved affinity purification method with an additional convenient purification tool, as noted by McCoy et al, which can be used for increasing the production of soluble recombinant proteins and satisfactorily

purifying them." (underlining added for emphasis, p. 8, Office Action, Paper No. 6)

First, Applicants note that the statement at column 4, lines 22-24 of McCoy, referenced by the Examiner, is taken from the "Summary of the Invention" and actually reads:

> "As yet another aspect, there is provided a novel method for increasing the production of soluble recombinant proteins."

Applicants respectfully submit that the proper meaning of the above-quoted statement from McCoy is that it is a general description of one of several embodiments of the invention that McCoy purports to disclose in which host cells are cultured under suitable conditions to produce certain fusion proteins (see, col. 4, lines 24-26 of McCoy). Accordingly, the statement is not a teaching, suggestion, or contemplation of a method *other than* that disclosed in McCoy and clearly does not provide a suggestion or motivation for being combined with the teachings of Zhang, which uses multiple nucleic acid probes to detect target nucleic molecules, to make Applicants' claimed improvements in affinity procedures that reduce particle loss.

Furthermore, even if the methods of McCoy for producing certain fusion proteins are combined with the multi-probe nucleic acid detection methods of Zhang, the result is a collection of two completely different methods for two completely different molecules, i.e., detection of target nucleic acids (Zhang) and expression of certain fusion proteins (McCoy). The combination of Zhang and McCoy does not bring a person of ordinary skill in the art any closer to appreciating or solving the problem of particle loss in affinity procedures by application of detergent, as claimed by Applicants. Thus, a person of ordinary skill in the art who reads Zhang and McCoy together still does not arrive at Applicants' claimed methods of improving affinity procedures by using a detergent prior to or contemporaneously with a step in which the affinity particles are manipulated. Accordingly, Applicants respectfully submit that the combination of Zhang and McCoy does not make out a *prima facie* case of obviousness to reject the claims.

Zhang and Gallant

Gallant describes a cysteine proteinase called apopain, which appears to play a key role in promoting apoptosis, and peptidyl derivative compounds that inhibit apopain (see, e.g., col. 1, lines 6-15; col. 10, lines 17-col. 14, line 56 of Gallant). Apopain cleaves the DNA repair enzyme poly (ADP-ribose) polymerase (PARP) in the early onset of apoptosis (see, e.g., col. 2, lilnes 36-47 of Gallant).

The Examiner relies on Gallant to provide a teaching of the use of the zwitterionic detergent CHAPS in an HPLC affinity purification method at col. 22, line 33-col. 23, line 27. The section of Gallant cited by the Examiner describes a purification scheme for apopain from

the human monocytic leukemic cell line THP-1. In particular, the purification scheme involves applying a cytosolic fraction of THP-1 cells to a DEAE-5PW HPLC column that had been pre-equilibrated in a Tris/HCl buffer comprising 0.1% (w/v) CHAPS zwitterionic detergent. Proteins were then eluted with a linear gradient of NaCl in Tris/HCl buffer also comprising 0.1% (w/v) CHAPS.

However, the method of Gallant is an HPLC column method and not an affinity method in which particles are manipulated and subject to loss during the manipulation. In particular, a person of ordinary skill in the art would understand that the HPLC column method used in Gallant is distinctly different from Applicants' claimed methods of using affinity particles which clearly comprise steps in which the particles are manipulated, e.g., collecting the affinity particles, separating the affinity particles with bound molecules from unbound components of a sample, resuspending the particles in solution (as in a wash step), or eluting and separating molecules of interest from the affinity particles to which they were bound. Applicants' claimed methods contain steps that require a manipulation of the affinity particles that possess the very real opportunity to lose significant amounts of particles and thereby affect yield (see, e.g., Examples 1-6 of the instant application). In contrast, the anion exchange particles of the HPLC set up in Gallant are never manipulated during use, but are physically contained, and therefore, are never at risk of being lost during the procedure. Hence, Gallant provides no recognition or insight into the problem addressed and solved by the improved affinity methods claimed by Applicants.

In addition, Gallant is devoid of any suggestion or motivation to be combined with Zhang to make Applicants' methods. Furthermore, even if Zhang is combined with Gallant, the result is the combination of two very different methods used to achieve two different purposes: a standard HPLC method to purify apopain proteinase and a multi-probe method of detecting target nucleic acid molecules. Clearly, Gallant adds nothing to advance the teachings of Zhang to arrive at Applicants' claimed methods. Accordingly, Applicants respectfully submit that the combination of Zhang and Gallant does not make out a *prima facie* case of obviousness to reject Applicants' claims.

Zhang and Stein

The method of Stein is an improved continuous process for separating mixtures of fatty alcohols that relies on manipulating fatty alcohol chemistry, including converting the fatty alcohol components in a mixture into different forms that have different melting points and then separating the subsequently formed liquid and solid forms of the converted fat compounds using aqueous wetting agent solutions (see, e.g., col. 3, line 55-col. 4, line 2 of Stein). Any of variety of

wetting agents may be used in the method of Stein (see, e.g., col. 5, line 59-col. 6, line 24 of Stein). The Examiner relied on Stein as a teaching for the use of the cationic detergent dodecyl trimethyl ammonium chloride (see, e.g., col. 6, lines 22-24). However, Stein does not utilize an affinity method. Thus, Stein provides no recognition of the problem addressed by Applicants' claimed invention. Furthermore, Stein provides no suggestion or motivation to be combined with Zhang to suggest Applicants' claimed affinity methods, which use detergent in conjunction with affinity particles to reduce particle loss during steps in which the particles are manipulated.

As with the other combinations set forth by the Examiner, even if Zhang is combined with Stein, the result is a confusing mixture of methods of using multiple nucleic acid probes to detect target nucleic acid sequences and a continuous process of separating fatty alcohols that relies on manipulating the chemistry of fat compounds. Thus, even when combined, the result of Zhang and Stein is not Applicants' claimed method for improving affinity procedures by using detergent to reduce loss of affinity particles. Clearly, Stein cannot cure the deficiency of the primary reference Zhang to make Applicants' claimed methods *prima facie* obvious, and accordingly reconsideration and withdrawal of the rejection based on Zhang and Stein is solicited.

Based on the above comments and the amendments to the claims, Applicants respectfully submit that none of the references, alone or in the combinations set forth in the Office Action, renders Applicants' claims *prima facie* obvious. Accordingly, reconsideration and withdrawal of the rejections under 35 U.S.C. § 103(a) are respectfully requested.

In view of the amendments to the claims and all of the above comments, Applicants submit that the Examiners' rejections have been avoided or overcome. Accordingly, Applicants respectfully request that the Examiner enter the amendments to the claims, withdraw the rejections, and pass the claims to allowance.

Respectfully submitted,

Thomas R. Berka, Ph.D., Reg. No. 39,606
Leon R. Yankwich, Reg. No. 30,237
Attorneys for Applicants
YANKWICH & ASSOCIATES
130 Bishop Allen Drive
Cambridge, Massachusetts 02139
telephone: (617) 491-4343
telefax: (617) 491-8801

CERTIFICATE OF MAILING

The undersigned hereby certifies that the items of correspondence referred to above are being deposited with the U.S. Postal Service as First Class mail under 37 C.F.R. §1.8, postage prepaid, in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231 on the date indicated below:

August 28, 2000
date of mailing and signature

Stephanie L. Pytlak
Stephanie L. Pytlak